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Research Article

Green Synthesis of Gold Nanoparticles using Aqueous Garlic (*Allium sativum L.*) Extract and Its Interaction Study with Melamine

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Abstract

Gold nanoparticles (AuNPs) have been successfully prepared by green synthesis method using aqueous extract of garlic with the Latin name of *Allium sativum L.* (ASL) as a reducing and stabilizing agents. Identification of active compounds in aqueous ASL extract was conducted by phytochemical analysis and Fourier transform infrared (FTIR) spectroscopy, while the synthesized AuNPs were characterized using UV-Vis spectrophotometer and transmission electron microscopy-selected area electron diffraction (TEM-SAED). The AuNPs formation was optimized at aqueous ASL extract concentration of 0.05%, $HAuCl_4$ concentration of 2.0×10^{-4} M, and pH of 3.6. The optimized AuNPs was characterized using TEM, and has a spherical shape with particle size of 15±3 nm. The particles were also stable up until one month. The synthesized AuNPs has been studied its interaction with melamine, and showed the optimum pH of interaction at 3.6.

Keywords: Green synthesis; Gold nanoparticles; *Allium sativum L*; Melamine

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1. Introduction

Green synthesis of nanoparticles has been a new trend in a safer and a more environmental friendly nanotechnology development. The green synthesis method does not rely on hazardous chemical agents, instead it relies on biological agents such as bacteria, plant extracts, fungi [1], and algae [2]. One of the advantages of green synthesis is the economic efficiency of process, in which the method requires relatively cheap materials. Other advantage is the simplicity of method due to one step process [3]. Several researches about green synthesis have been done before used various plant extracts due to their simple preparation. Various plant extracts used in the previous researches are *Ficus religiosa* [4], *Memecylon umbellatum* [5], *Macrotyloma uniflorum* [6], *Brevibacterium casei* [7], honey [8], *Citrus limon*, *Citrus reticulate*, *Citrus sinensis* [9], *Piper pedicellatum* [10], *Terminalia chebula* [11], *Nyctanthes arbortristis* [12], *Murraya Koenigii* [13], *Mangifera indica* [14], *Cinnamomum zeylanicum* [15], *Cochlospermum gossypium* [16], and *Zingiber officinale* [17].

Nanoparticles from noble metals such as gold, silver, and platinum have been utilized in numerous applications such as in medicines, cosmetics, biological sensors, and catalysts.

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These are due to the noble metal nanoparticles have large surface areas [18-22]. Gold nanoparticles (AuNPs) have certain characteristics and properties those can be applied in biomedics, industries, drug delivery agents, and sensors. AuNPs has several advantages compared other nanoparticles namely inert, resistance to oxidation, biologically nonhazardous and compatible [23]. AuNPs has a unique surface plasmon resonance [24] due to its ability to absorb a visible light, so it is often used as an ideal colorimetric sensor.

In this research, AuNPs was synthesized using aqueous extract of *Allium sativum L.* (ASL) as a reducing and stabilizing agents. ASL is herbal plant that thrives in Indonesian plantations, and contains 100 beneficial secondary metabolites for the human body. Previous research reported that ASL extract can be utilized as a reducing and stabilizing agents in the synthesis of silver nanoparticles (AgNPs) [25]. There have never been any reports about the use of ASL extract in the green synthesis of AuNPs as an alternative route for an environmental friendly synthesis.

The synthesized AuNPs from this research was observed its colorimetric system, which has the potency to detect a melamine through its interaction study. Melamine as an illegal food additive has inert and toxic properties [26]. The common recent melamine detection methods includes high performance liquid chromatography (HPLC), mass spectrometry (MS), gas chromatography/mass spectrometry (GC/MS), high performance liquid chromatography/mass spectrometry (HPLC/MS), capillary zone electrophoresis/mass spectrometry (CE/MS), surface enhanced Raman spectoscopy (SERS) [27,28]. The all mentioned methods have high sensitivity, however require long time and expensive preparations. Therefore, AuNPs in this research was prepared by green synthesis method for the first time using aqueous ASL extract. Its potency was investigated as a cheap, fast and simple melamine colorimetric detector through their interaction study, and the fact of melamine is able to induce AuNps aggregation that causes a color change in the visible area [29].

2. Materials and Methods

2.1. Materials

Allium sativum L. (ASL) was obtained from Alam Endah Village at Rancabali district, Bandung, Indonesia. Melamine was obtained from Merck. Methanol, n-hexane, n-butanol, and ethyl acetate were obtained from PT. Bratako. $HAuCl_4$ used in this research was synthesized by dissolving 99.9 % Au metal from PT. Antam in aqua regia solvent with HNO_3 and HCl ratio of 1:3. All chemicals were analytical grade and used without further purification. MilliQ water (18.2 $W.cm^{-1}$) was used to make aqueous solutions.

2.2. Preparation of *Allium sativum L.* extract

About 1.5 kg of ASL was sliced to thin layers and dried in room temperature for one week. The dried layers were blended to soft powders. The ASL powder of 60 g was macerated in 300 mL of bi-distilled water for five days, and mixed constantly. The macerated ASL was filtered and the filtrate was extracted using n-hexane in a separation funnel until it formed two fractions. The obtained water fraction was re-extracted using ethyl acetate and n-butanol, respectively. The separated water fraction was phytochemicals analyzed of its polyphenol, tannin, alkaloid, flavanoid, saponnin, terpenoid, and steroid contents.

The water fraction was saturated using vacuum rotary evaporator to remove its water solvent and characterized using FTIR spectroscopy (Shimadzu Prestige 21). The saturated ASL extract was then made into a stock solution with 30 % concentration (v/v). The stock solution was prepared to various concentration of ASL extract for the synthesis of AuNPs.

2.3. Synthesis of Au nanoparticles using various concentration of ASL extract

About 10 mL of 1.0×10^{-4} M $HAuCl_4$ solution was added 0.01-0.11 % (v/v) aqueous ASL extract under UV lamp radiation for two hours. The biotransformation of $AuCl_4^-$ to AuNPs was constantly observed from the color changes and monitored using UV-Vis spectrophotometer (Shimadzu 2600).

2.4. Synthesis of Au nanoparticles using various concentration of $HAuCl_4$

Synthesis of AuNPs using various concentration of $HAuCl_4$ was done by reacting ASL extract at optimum concentration (0.05 %) in 10 mL $HAuCl_4$ with concentrations: 5.0×10^{-5}; 1.0×10^{-4}; 2.0×10^{-4}; and 3.0×10^{-4} M under UV light radiation for two hours. The results were observed through the color changes and measured using UV-Vis spectrophotometer.

2.5. Synthesis of Au nanoparticles against pH variation

Synthesis of AuNPs against pH variation was conducted by reacting 10 mL $HAuCl_4$ solution at optimum concentration 2.0×10^{-4} M and ASL extract 0.05 % at pH variation of 1.3; 2.7; 3.6; 4.6; 5.5; 6.5; 7.4; and 8.3 under UV light radiation for 2 hours. HCl and NaOH solutions with concentrations of 2.0×10^{-2} M each were used to varying the pH of reaction. The reaction was observed from the color change and measured using UV-Vis spectrophotometer. After the optimum pH was achieved, the synthesized AuNPs were then characterized using FTIR spectroscopy and TEM-SAED (JEM 1400).

2.6. Interaction study of AuNPs with melamine

About 0.5 mL of melamine with concentration 1.0×10^{-5} M was added into the synthesized AuNP colloid under optimum conditions (concentration and pH), and stirred by magnetic stirrer. The absorption spectrum of reaction was then measured using UV-Vis spectrophotometer and studied its interaction.

3. Results and Discussion

3.1. Identification of aqueous ASL extract

Identification of aqueous ASL extract was firstly done by phytochemical test. It is conducted to qualitatively analyze the presence of secondary metabolites in aqueous ASL extract. The result showed that aqueous ASL extract positively contained polyphenol and terpenoids which are biologically active compounds [30], therefore their present play a major role in the synthesis of AuNPs.

The FTIR spectrum of aqueous ASL extract (Figure 1) at wavenumber of 3250 cm^{-1} shows the presence of O–H stretching vibration in hydroxyl group, 2920 cm^{-1} (presence of asymmetric stretching in C–H bonds), 1603 cm^{-1} (presence of carbonyl or carboxylic (C=O) stretching bands), 1407 cm^{-1} (presence of –O–H bend in carboxylic), 1122 cm^{-1} (presence of S=O bond), 1019 cm^{-1} (presence of C–N stretching vibrations in primary amines), 934 cm^{-1} (presence of γ-C–H deformation in $=CH_2$), 815 cm^{-1} (presence of N–H bend in primary amines) and 530 cm^{-1} (presence of C–H bend in alkynes). These FTIR spectrum results were very similar to the previous researcher studied about the functional groups presence in aqueous ASL extract [31]. Based on the acquired spectrum, the active groups that play a role in the reduction and the stabilization of AuNPs are phenolic, organosulfur compounds, amino acids, carboxylic groups, and proteins [32].

3.2. Effect of various ASL extract concentration on AuNPs synthesis

Synthesis of AuNPs was conducted by reducing the monovalent or trivalent Au ions to uncharged Au atoms. In this research, synthesis of AuNPs was done by a one step process, which was by reacting aqueous ASL extract with $HAuCl_4$ solution under the exposure of UV light. Visual observations shows that the synthesis of nanoparticles by various extracts

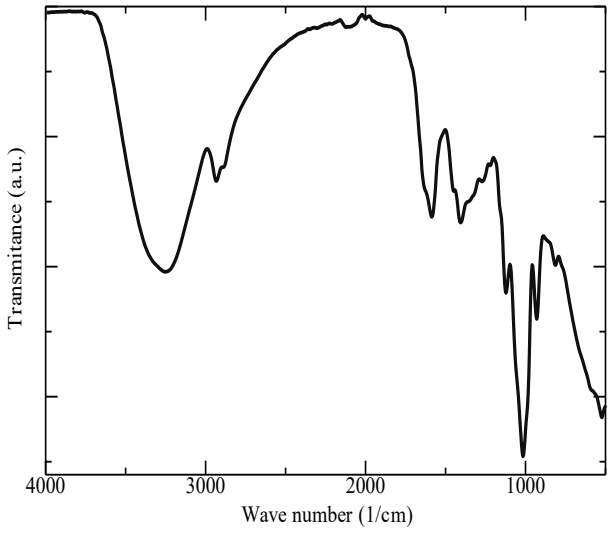

Figure 1. FTIR spectrum of aqueous ASL extract

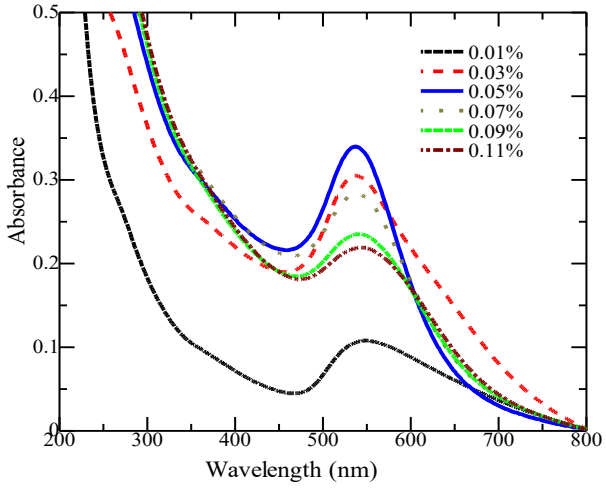

Figure 2. UV-Vis absorption spectra of AuNPs prepared using various concentrations of aqueous ASL extract at 1.0×10^{-4} M $HAuCl_4$ under UV lamp radiation

concentration affect the color of nanoparticles. The produced AuNPs have a bright red color in a colloidal system with absorbance range of 510-550 nm [33-35]. Observations using UV-Vis spectrophotometer of the synthesized AuNPs were used to determine the optimum AuNPs formation through the change of wavelength and absorbance (Figure 2).

The color of colloid particles produced using 0.05 % of ASL extract concentration was bright red with higher and sharper absorbance in comparison with the other particle absorbance, at λ_{max} = 538 nm. Therefore, the optimum condition of AuNPs synthesis was determined at 0.05 % of ASL extract concentration. The pattern of UV-Vis absorption spectrum correlated with the nanoparticle size polydispersity. The sharpness of spectrum indicates the homogeneity of particles sizes [8,36].

3.3. Effect of various $HAuCl_4$ concentration on AuNPs synthesis

The optimum concentration of aqueous ASL extract (0.05 %) was used to determine the optimum concentration of $HAuCl_4$ solution for AuNPs synthesis. Based on the observation, the higher concentration of $HAuCl_4$ produced the darker color of colloid particles. This happened possibly due to at a very high concentration of $HAuCl_4$ solution, the core of AuNPs was produced denser and easily agglomerated gave the nanoparticles darker color. The bright red color of AuNPs formation was achieved at $HAuCl_4$ solution concentration of 2.0×10^{-4} M, with the maximum absorption wavelength, λ_{max} at 538 nm. Therefore, it can be concluded that $HAuCl_4$ solution concentration of 2.0×10^{-4} M is the optimum concentration of AuNPs synthesis as shown in Figure 3.

3.4. Effect of pH on AuNPs synthesis

Besides precursors and reducing agent in the nanoparticles synthesis, pH plays a significant role in the nanoparticles formation. The obtained optimum pH from this experiment of AuNPs synthesis was 3.6 as shown in Figure 4. The reversible chemical reaction of AuNPs formation at pH = 3.6 can be written as below:

$$HAuCl_4 + H_2O + 3e^- \leftrightarrow Au^0 + H_3O^+ + 4Cl^-$$

In this experiment, the AuNPs was easily formed at alkaline condition. In a highly acidic condition (pH=1.3), the concentration of H_3O^+ increases and shifts the chemical reaction to reactant side as correspond to the Le' Chatelier principle. In a visual observation, the reactant side to be colorless. In conclusion, the Au^0 species are barely formed at highly acidic condition. The opposite phenomenon will happen at alkaline condition. The concentration of H_3O^+ decreases as the species are consumed by the OH^- ions, thus shifting the reaction to the product side. In this experiment however, the formation of AuNPs can be halted by too much OH^- ions due to have lone paired electrons (LPE) that may form a complex with Au ions from $HAuCl_4$.

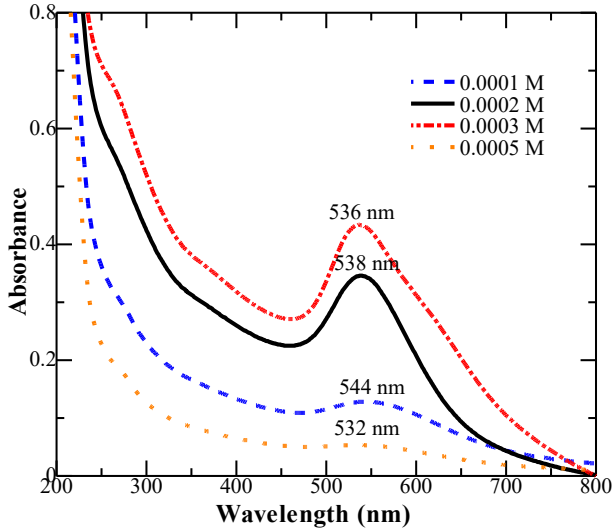

Figure 3. UV-Vis absorption spectra of AuNPs prepared using 0.05 % aqueous ASL extract at various $HAuCl_4$ concentration under UV lamp radiation.

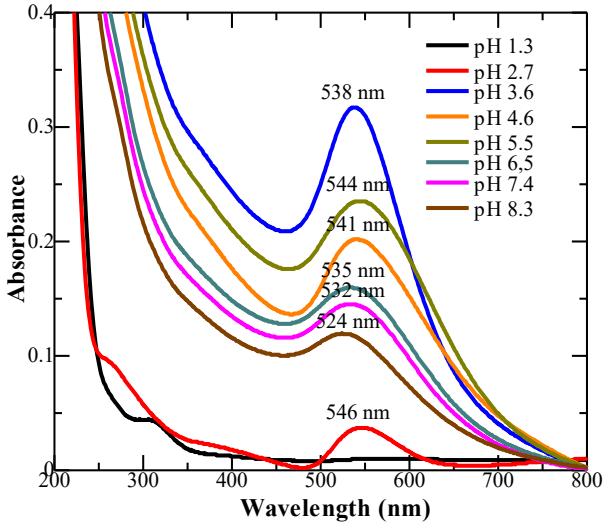

Figure 4. UV-Vis absorption spectra of AuNPs prepared using 0.05 % aqueous ASL extract and $2.0\text{x}10^{-4}$ M $HAuCl_4$ at various pH under UV lamp radiation

3.5. Analysis of AuNPs using TEM-SAED

The morphological and crystalline analysis of AuNPs was done using TEM-SAED at optimum condition of $HAuCl_4$ concentration 2.0×10^{-4} M, aqueous ASL extracts concentration 0.05 %, and pH 3.6 under UV light radiation. Aqueous ASL extract was not able to reduce Au^{3+} to Au^0 at room temperature, therefore UV light was needed to provide enough energy to release the electrons from ASL extract to reduce it. The synthesized AuNPs at optimum condition was stable for one month. TEM characterization showed homogenously dispersed AuNPs with relatively small spherical shapes (15±3 nm) as shown in Figure 5. The homogenously dispersed and controlled nanoparticle size due to the aqueous ASL extract played a role also as a good stabilizer of AuNPs through capping its surface. The TEM-SAED pattern in Figure 6 showed that AuNPs formed a crystalline structure of face-centered cubic (FCC) with Miller index (111), (200), (220), (311), (222), and (400), in correlation to JCPDS Au No. 04-0784.

3.6. Interaction study of AuNPs with melamine

In this experiment, the interaction of AuNPs with melamine was studied using UV-Vis spectrophotometer through the absorbance value change and maximum wavelength, λ_{max} shift from its absorption spectra. The pH of reaction was varied to further investigate its interactions with melamine.

The changes of absorbance and λ_{max} of AuNPs, before and after the addition of melamine under various pH are shown in Table 1. The change in λ_{max} was observed at pH 3.6 and 4.6. At pH bellow 3.6, there was a great change of them ($\Delta\lambda_{max}$ and ΔAbs). This was not due to a strong interaction between AuNPs and melamine, however its stabilizing agent being protonated, and released from its surface caused AuNPs aggregation. The aggregated AuNPs has a larger particle size that caused a shift in λ_{max} and a decrease in absorbance. At pH greater than 4.6, there was no change in λ_{max} due to an increasing amount of OH^- ions. The increase of OH^- ions caused a disturbance of interaction between AuNPs and melamine. Therefore the interaction of AuNPs with melamine was occurred at pH of 3.6-4.6.

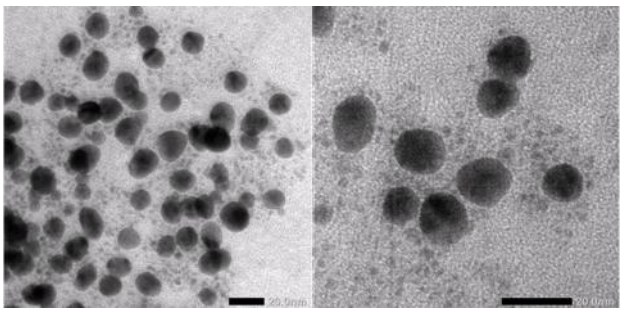

Figure 5. TEM images of green synthesized AuNPs

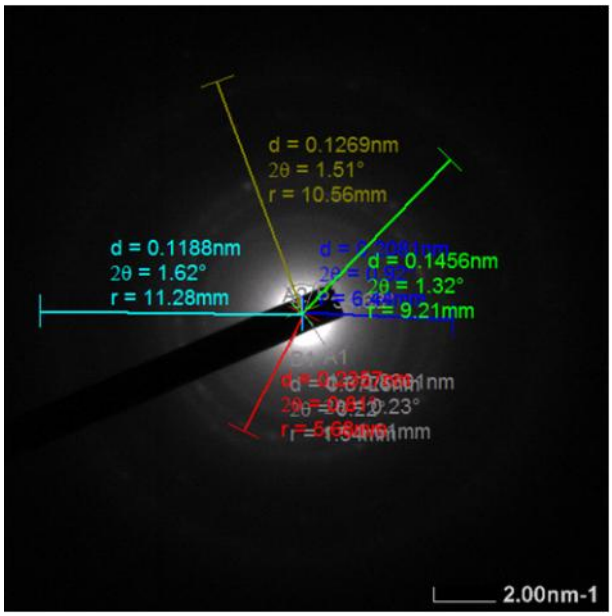

Figure 6. Selected area electron diffraction pattern of green synthesized AuNPs

Table 1. The changes of absorbance and λ_{max} of AuNPs, before and after the addition of melamine at various pH

	Initial AuNPs	pH 2.3	pH 3.6	pH 4.6	pH 5.7	pH 6.5	pH7.7	pH 8.4
λ_{max}	535	545	537	537	535	535	535	535
$\Delta\lambda_{max}$	-	10	2	2	0	0	0	0
Abs	0.334	0.028	0.318	0.311	0.31	0.312	0.311	0.305
ΔAbs	-	0.306	0.016	0.023	0.024	0.022	0.023	0.029

Abs = Absorbance, Δ Abs = Absorbance change

4. Conclusions

The AuNPs was successfully synthesized using aqueous extract of garlic, *Allium sativum L.* (ASL) by an alternative environmental friendly method of metal nanoparticles formation. AuNPs synthesis was optimized using aqueous ASL extract concentration of 0.05 %, $HAuCl_4$ concentration of 2.0×10^{-4} M, and pH of 3.6 characterized using UV-Vis spectrophotometry, FTIR spectroscopy, and TEM-SAED. The synthesized AuNPs had a good homogeneity, which showed the aqueous ASL extract act as a good reducing and stabilizing agents. The structure and crystalline of AuNPs were analyzed from SAED pattern, showed that AuNPs crystalline was face-centered cubic. Green synthesized AuNPs showed a good interaction with melamine.

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